Establishment of Baseline Susceptibility Data to Various Insecticides for *Homalodisca coagulata* (Homoptera: Cicadellidae) by Comparative Bioassay Techniques

NILIMA PRABHAKER,[1] STEVEN CASTLE,[2] FRANK BYRNE,[1] T. J. HENNEBERRY,[2] AND NICK C. TOSCANO[1]

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ABSTRACT *Homalodisca coagulata* Say , adults from three locations in California were subjected to insecticide bioassays to establish baseline toxicity. Initially, two bioassay techniques, petri dish and leaf dip, were compared to determine the most useful method to establish baseline susceptibility data under laboratory and greenhouse conditions. Comparative dose-response data were determined by both techniques to endosulfan, dimethoate, cyfluthrin, and acetamiprid. Toxic values were similar to some insecticides with both techniques but not for all insecticides, revealing susceptibility differences among the three populations of *H. coagulata.* In subsequent tests, the petri dish technique was selected to establish baseline susceptibility data to various contact insecticides. A systemic uptake bioassay was adapted to estimate dose-mortality responses to a systemic insecticide, imidacloprid. A 2-yr comparison of toxicological responses showed all three populations of *H. coagulata* to be highly susceptible to 10 insecticides, including chlorpyrifos, dimethoate, endosulfan, bifenthrin, cyfluthrin, esfenvalerate, fenpropathrin, acetamiprid, imidacloprid, and thiamethoxam. In general, two pyrethroids, bifenthrin and esfenvalerate, were the most toxic compounds, followed by two neonicotinoids, acetamiprid and imidacloprid. The LC_{50} values for all insecticides tested were lower than concentrations used as recommended field rates. Baseline data varied for the three geographically distinct *H. coagulata* populations with the petri dish technique. Adult *H. coagulata* collected from San Bernardino County were significantly more susceptible to select pyrethroids compared with adults from Riverside or Kern counties. Adults from San Bernardino County also were more sensitive to two neonicotinoids, acetamiprid and imidacloprid. The highest LC_{50} values were to endosulfan, which nonetheless proved highly toxic to *H. coagulata* from all three regions. In the majority of the tests, mortality increased over time resulting in increased susceptibility at 48 h compared with 24 h. These results indicate a wide selection of highly effective insecticides that could aid in managing *H. coagulata* populations in California.

KEY WORDS *H. coagulata*, neonicotinoids, organophosphates, petri dish bioassay, systemic bioassay

Homalodisca coagulata Say became a pest of economic importance in many areas of southern California during the 1990s (Blua et al. 1999). It is a principal vector of *Xylella fastidiosa*, a plant pathogenic bacterium that causes Pierce's disease (PD) in grapes (Purcell 1981). *H. coagulata* is also known to transmit other strains of *X. fastidiosa* that cause oleander leaf scorch in oleander and almond leaf scorch in almonds (Blua et al. 1999). Effective management of the vector is essential to control the spread of PD and other strains of *X. fastidiosa.* Insecticides are among the many control options currently available for managing *H. coagulata.* Select insecticides that have been used to suppress *H. coagulata* populations seem to have been very effective (Akey et al. 2001). Both the Environmental Protection Agency (EPA) and California registrations for certain conventional insecticides as well as for more selective newer insecticides have been amended recently to include *H. coagulata* on citrus and grapes.

Successful implementation of insecticides in management programs of this pest requires a careful evaluation of the efficacy of available chemicals. Development of resistance is always a potential risk when insecticides are used in pest management. Metcalf (1975) pointed out that insecticides are the primary tools for remedial action when insect pest populations exceed the economic threshold, and this remains true today for *H. coagulata.* Therefore, efforts should be made to evaluate and recommend the most effective insecticides for *H. coagulata* control.

The first step in examining the effectiveness of insecticides against *H. coagulata* populations and in identifying potential problems that might arise be-

[1] Department of Entomology, University of California, Riverside, CA 92521.
[2] Western Cotton Research Laboratory, USDA–ARS, Phoenix, AZ 85040.

cause of resistance is to monitor for baseline susceptibility. Knowledge of baseline susceptibility responses of *H. coagulata* populations to insecticides is essential for keeping track of changes in toxicological responses of the pest over time. These baseline data can be used as reference values to diagnose shifts in susceptibility and promote alternative chemistry or control practices if resistance increases. Potentially heavy reliance on insecticides to manage this pest can lead to shifts in susceptibility as observed in numerous other pests. Additionally, monitoring results can give comparisons for presence of any cross-resistance patterns between different classes of insecticides. Establishment of baseline data could aid in future development of an effective *H. coagulata* integrated pest management (IPM) program.

The purpose of the present investigation was to assess and monitor the susceptibility of *H. coagulata* populations to various insecticides as part of the *H. coagulata* management program for several reasons. First, no susceptibility data were available on *H. coagulata* responses to insecticides at the time it became established in southern California. Insecticides that were applied against this pest were recommended for other pests on grapes and citrus. Therefore, monitoring was necessary in this case to establish a database of baseline susceptibility of different populations of *H. coagulata* before widespread applications of insecticides were implemented. Generally, monitoring should be started early-on in a chemical control program by testing field samples for comparison of susceptibilities to insecticides under controlled conditions before their extensive use. Bethke et al. (2001) examined the effects of fenpropathrin, fenpropathrin + acephate, acetamiprid, imidacloprid, and thiamethoxam on *H. coagulata* mortality and feeding behavior in relation to oleander leaf scorch disease transmission under greenhouse conditions that provided limited toxicological data. Second, monitoring responses to insecticides will aid in understanding the nature of resistance if it develops in this pest. Potential changes in responses detected in subsequent tests can be compared against the baseline data to provide a better understanding of the relative severity of the resistance, especially if LC_{50} values are increasing. Comparison of susceptibility shifts to specific compounds or chemical classes may help to identify the scope and nature of the resistance mechanism. Third, it was critical to identify the most effective products for use in *H. coagulata* chemical control programs. The bioassay data obtained in the laboratory could offer immediate practical guidance to individual growers in lieu of conducting expensive trials to determine the effectiveness of a particular insecticide. Identification of a range of effective products would provide the growers greater diversity in the choice of chemicals and help to avoid undue selection pressure on one or two chemicals. Therefore, the relative toxicities of 10 insecticides that represented various classes of chemistries were tested against *H. coagulata.* Geographically discrete populations of *H. coagulata* from San Bernardino, Riverside, and Kern counties were assayed to determine comparative baseline susceptibility levels for the first year. The three geographically separate regions in California were selected based on presence of heavy populations on citrus in their respective regions. Follow-up bioassays on populations from the same areas were conducted the following year to measure the possible changes in responses of populations so that any significant shifts in baseline responses over time can be used for formulating resistance management strategies for this pest.

The initial step for monitoring responses of *H. coagulata* populations is through establishment of appropriate bioassay techniques that provide consistent baseline susceptibility data. Techniques should be sufficiently sensitive to detect the low frequency of resistance genes that are likely to be present initially in *H. coagulata* populations. Once a technique has been standardized and found to be feasible for use, it can be used in resistance monitoring programs for *H. coagulata.* Resistance monitoring is essential to all resistance management programs (Roush and Miller 1986). The present report compares the baseline data of a number of *H. coagulata* populations from southern California to four contact insecticides, endosulfan, dimethoate, cyfluthrin, and acetamiprid, by two techniques, petri dish and leaf dip methods. Based on the results, one technique, the petri dish bioassay, was selected to establish baseline susceptibility data to five more contact insecticides that are used for pests on citrus and grapes. Baseline susceptibility data to imidacloprid, a systemic insecticide, was established by a systemic uptake bioassay technique. Tests were conducted over 2 yr. The appropriate choice of bioassays will enable changes in susceptibility to insecticides to be detected and lead to more effective resistance management as well as long-term resistance monitoring.

Materials and Methods

Collection of Insects. Several field collections of *H. coagulata* from the three California locations, including San Bernardino, Riverside, and Kern counties were made from both untreated and insecticide-treated commercial vineyards and citrus orchards for monitoring purposes. The majority of the collections were made from June 2001 to June 2002 and from July 2002 to June 2003. Collections of *H. coagulata* populations were made on the starting day of each bioassay using sweep net and bucket sampling devices from citrus orchards (Castle et al. 2005). Insects were placed in plastic bags and transported to the laboratory or greenhouse for bioassays. Plastic bags holding collections of insects from Kern and San Bernardino counties were held in closed cooler chests en route to minimize stress during the long trip back to the laboratory in Riverside. In the laboratory, plastic bags with *H. coagulata* were placed in transfer cages containing citrus seedlings to allow the insects to transfer out of each bag to citrus for feeding. Insects that were actively feeding on citrus were selected for each test to avoid unacceptable levels of control mortality.

Table 1. Insecticides tested against *H. coagulata*

Class	Compound	Registered name	Company
Organophosphate	Chlorpyrifos	Lorsban	DowElanco, Indianapolis, IN
	Dimethoate		BASF, Florham, NJ
Pyrethroid	Bifenthrin	Capture	FMC Corp., Philadelphia, PA
	Cyfluthrin	Baythroid	Bayer, Kansas City, MO
	Esfenvalerate	Asana	DuPont Agricultural Products, Wilmington, DE
	Fenpropathrin	Danitol	Valent USA Corp., Walnut Creek, CA
Cyclodiene	Endosulfan	Thiodan	FMC Corp.
Neonicotinoid	Acetamiprid	Assail	DuPont Agricultural Products
	Imidacloprid	Admire	Bayer
	Thiamethoxam	Actara	Syngenta, Oxnard, CA

Insecticides. Adults were tested for susceptibility to selected insecticides. Bioassays were conducted using 10 insecticides, nine with contact and one with systemic activity, all selected to represent various classes of insecticides (Table 1). Formulated materials were used in all cases. At least five concentrations of each insecticide were used to obtain mortality that ranged from 5 to 95%. Serial dilutions with water were made on the day of testing.

Bioassay for Contact Insecticides. Two types of bioassay techniques, petri dish and leaf dip, were adapted and modified to assess the susceptibility of field populations of *H. coagulata* to various contact insecticides.

Petri Dish Bioassay. The petri dish bioassay was adapted from previous studies (unpublished data) using disposable 60-mm petri dishes. Agar beds (1.5%) were prepared in each petri dish for maintenance of freshly cut citrus leaf disks for up to 1 wk. Excised citrus leaf discs (60 mm) were dipped for 30 s in five concentrations of each insecticide and allowed to dry for an hour before placing them on the agar beds. Preliminary dosages were determined before commencement of tests for the selected insecticide to establish mortality ranging from 5 to 95% mortality. Water-treated leaf discs served as controls for each set of treatments. For exposure to the treatments, adults were briefly (5–7 s) anesthetized in plastic vials with carbon dioxide and then transferred to petri dishes in lots of five to 10 by tapping them onto each treated leaf in the petri dish. Handling insects by using carbon dioxide was found to be safe to insects and kept the control mortality below 5% consistently. At least five replications of each concentration were included per bioassay for each insecticide. Each bioassay was replicated at least three times. Experimental treatments were evaluated by recording *H. coagulata* mortality at 24- and 48-h intervals. In the case of acetamiprid and thiamethoxam, mortality was assessed after 12 and 24 h followed by 48 h because of their greater potency to the insects. Insects were maintained at 27 ± 2°C and a photoperiod of 14:10 (L:D) h in petri dishes. Any adult that failed to move when touched or was immobile on its back was considered dead.

Leaf Dip Bioassay. Leaf dip bioassays of attached leaves on citrus plants were conducted in the greenhouse. Five serial dilutions of endosulfan, dimethoate, cyfluthrin, and acetamiprid were used for dipping. Five replications of each concentration were included per bioassay, and each bioassay was repeated three times. Terminal leaves on multiple branches of potted citrus plants were immersed for 30 s in each concentration. The dipped leaves were air-dried for an hour. At least five adults were placed in 7.0-cm^2 clip cages and attached to the treated leaves for exposure. Mortality assessment was similar to that of the petri dish bioassay described above.

Bioassay for Systemic Insecticide. To assess the toxicity of imidacloprid through systemic exposure, a system was developed that allowed detached citrus leaves to take up imidacloprid through their petioles (Prabhaker et al. 2005). Excised stems with two terminal leaves were placed in serial dilutions of imidacloprid in aquapiks for 24 h. After the uptake period, leaves were transferred to aquapiks containing water only. Adult *H. coagulata* were exposed to the treated leaves in small clip cages similar to the attached leaf dip bioassay. At least five insects were enclosed per clip cage. Exposure time of the insects to imidacloprid was similar to that of the contact insecticides. Mortality was checked after 24 and 48 h.

Statistical Analysis. The LC_{50} and LC_{90} values expressed as nanograms per milliliter, 95% fiducial limits (FL), and slopes of the regression lines were estimated by probit analysis using POLO (Russell et al. 1977, LeOra Software 1987). Toxicity of endosulfan to *H. coagulata* was measured as micrograms per milliliter. Differences in LC_{50} values at 24- and 48-h exposures among *H. coagulata* collected from different locations to a particular insecticide were considered significant if there were no overlaps of 95% FL.

Results

Comparison of Bioassay Techniques to Establish Baseline Toxicity. Initial susceptibility of *H. coagulata* adults to four insecticides—endosulfan, dimethoate, cyfluthrin, and acetamiprid—was estimated by two comparative bioassay methods, petri dish and leaf dip techniques. Results showing comparison of *H. coagulata* responses to the four compounds by the two techniques are presented below (Tables 2–5). This part of our study was critical to enable selection of the most suitable and useful bioassay technique to establish baseline susceptibility data to chlorpyrifos, bifenthrin, esfenvalerate, fenpropathrin, and thiamethoxam. Toxicity results presented below contributed to the selection of the petri dish bioassay technique as a standard method for establishment of

Table 2. Comparison of toxicity of endosulfan to *H. coagulata* adults by petri dish and leaf-dip bioassays

Location (county)	Test method	Yr	Time	*n*	Slope ± SE	LC_{50} μg/ml (95% FL)	LC_{90} μg/ml (95% FL)	χ^2 (df)
San Bernardino	PD	2001	24	310	3.1 ± 0.26	65.90 (47.92–89.86)	221.66 (155.76–299.55)	4.87 (4)
			48		3.7 ± 0.03	29.95 (23.96–41.93)	107.83 (77.88–155.76)	9.85 (3)
	PD	2002	24	352	2.2 ± 0.25	16.97 (11.98–29.95)	71.89 (47.92–125.37)	3.64 (4)
			48		1.4 ± 0.20	1.81 (0.598–2.99)	11.98 (8.98–23.96)	2.86 (4)
	LD	2001	24	296	2.7 ± 0.19	71.89 (53.91–96.89)	227.65 (173.73–311.53)	2.72 (4)
			48		3.7 ± 0.22	51.72 (39.86–69.91)	149.77 (107.83–203.69)	2.64 (4)
	LD	2002	24	360	2.7 ± 0.12	69.38 (53.92–90.63)	207.65 (167.88–303.53)	1.89 (4)
			48		2.3 ± 0.46	47.93 (29.95–89.86)	127.41 (100.52–189.43)	3.54 (3)
Riverside	PD	2001	24	276	3.2 ± 0.34	53.81 (41.93–65.90)	184.68 (149.77–245.63)	2.63 (4)
			48		3.1 ± 0.39	11.98 (5.99–17.97)	35.94 (23.96–47.92)	1.66 (3)
	PD	2002	24	356	2.8 ± 0.19	121.66 (61.75–176.25)	254.51 (144.33–296.53)	2.16 (4)
			48		1.9 ± 0.29	41.93 (23.96–119.91)	120.96 (65.94–239.12)	2.72 (3)
	LD	2001	24	271	2.6 ± 0.15	59.91 (41.93–77.88)	197.70 (143.78–269.59)	4.65 (4)
			48		2.4 ± 0.40	47.92 (35.94–65.90)	167.74 (125.81–227.65)	2.68 (4)
	LD	2002	24	350	2.2 ± 0.13	50.92 (35.94–65.90)	179.73 (113.82–215.67)	3.04 (4)
			48		2.0 ± 0.10	11.98 (5.99–23.96)	65.90 (29.95–143.33)	2.63 (3)
Kern	PD	2001	24	285	3.4 ± 0.26	71.89 (47.92–89.58)	233.64 (167.74–282.29)	3.25 (4)
			48		2.5 ± 0.17	17.99 (13.92–28.89)	57.18 (44.79–84.10)	2.24 (3)
	PD	2002	24	366	1.4 ± 0.32	15.99 (2.39–31.98)	59.91 (23.96–89.86)	2.53 (4)
			48		1.9 ± 0.21	5.15 (3.19–9.21)	19.81 (15.39–39.95)	1.83 (3)
	LD	2001	24	222	2.6 ± 0.31	55.19 (42.91–79.76)	137.51 (92.31–164.50)	2.92 (4)
			48		2.7 ± 0.18	17.97 (5.99–35.39)	55.76 (38.87–64.20)	2.14 (3)
	LD	2002	24	280	2.7 ± 0.33	47.92 (23.96–65.90)	124.51 (91.51–143.24)	5.22 (4)
			48		2.8 ± 0.16	5.91 (4.29–11.98)	28.34 (19.17–35.71)	2.04 (3)

baseline data. Results of baseline susceptibility data using the petri dish technique are presented in the next section.

Endosulfan. Based on the LC_{50} and LC_{90} values obtained by the petri dish method (Table 2), *H. coagulata* adults proved to be very susceptible to endosulfan. Median lethal mortality was relatively equal in the three geographic populations at 24-h exposure with LC_{50} values ranging from 53.81 to 71.89 μg/ml in 2001. The LC_{50} values decreased significantly at 48 h posttreatment among the three populations based on nonoverlap of confidence limits (LC_{50} values ranging from 11.98 to 29.95 μg/ml).

In 2002, significant variation in susceptibility to endosulfan was observed. Insects from San Bernardino and Kern counties were more susceptible to endosulfan (ca. four-fold) after 24-h exposure compared with the previous year. By contrast, insects from Riverside County were 4 times less susceptible to endosulfan (LC_{50} = 121.66 μg/ml) at the 24-h observation. Variable increases in susceptibility were observed in all three populations after 48 h, by nine-fold for San Bernardino insects and three-fold for Riverside and Kern county insects (LC_{50} = 41.93 and 5.15 μg/ml, respectively) (Table 2).

Bioassay responses to endosulfan by the leaf dip method generated LC_{50} values similar to those obtained by the petri dish technique at the 24-h reading in 2001 (Table 2). Toxicity did not increase significantly with this bioassay method with longer exposure as observed by the petri dish technique in San Bernardino or Riverside populations, except for a threefold increase in Kern county insects. A notable difference in responses of the *H. coagulata* populations to endosulfan in 2002 was observed by the leaf dip technique. The LC_{50} values determined in 2002 were similar to those observed in the previous year with the petri dish technique in contrast to the lower LC_{50} values observed in 2002. These results suggest that responses of insects as measured by the petri dish technique indicated increased susceptibility to endosulfan in 2002, whereas no shifts in susceptibility occurred with the leaf dip technique. Slopes of the probit regression lines obtained with both bioassay techniques were fairly steep among the three *H. coagulata* populations, the highest recorded at 3.7 ± 0.23.

Dimethoate. Adults from Kern county were four- and five-fold less susceptible to dimethoate (LC_{50} = 43.78 ng/ml) at 24 h as determined with the petri dish technique (Table 3) than San Bernardino or Riverside populations (LC_{50} = 11.52 and 8.11 ng/ml, respectively). Toxicity increased similarly by 3 times in both San Bernardino and Riverside populations over time (48-h exposure) with this technique (LC_{50} = 3.43 and 2.61 ng/ml, respectively), but by only 2-fold for Kern county insects (20.33 ng/ml). However, this small increase in toxicity after 48 h in all three *H. coagulata* populations was significant (nonoverlap of 95% fiducial limits). Based on LC_{50} values, no significant shifts in responses of *H. coagulata* from the three regions to dimethoate were observed in 2002 as measured by the petri dish technique.

Although minor differences in susceptibility to dimethoate were observed in *H. coagulata* populations using the leaf dip technique, the overall trend was similar with both techniques (Table 3). Similar to the *H. coagulata* responses exhibited to dimethoate by the petri dish technique, both populations from San Bernardino (LC_{50} = 6.21 ng/ml) and Riverside county (LC_{50} = 11.34 ng/ml) were more sensitive to dimethoate by seven- and four-fold, respectively, than Kern county (LC_{50} = 46.37 ng/ml) adults. Toxicity did not increase significantly after 48-h exposure in San Bernardino and Riverside county populations. How-

Table 3. Comparison of toxicity of dimethoate to *H. coagulata* adults by petri dish and leaf-dip bioassays

Location (county)	Test method	Yr	Time	*n*	Slope ± SE	LC_{50} ng/ml (95% FL)	LC_{90} ng/ml (95% FL)	χ^2 (df)
San Bernardino	PD	2001	24	224	2.0 ± 0.31	11.52 (9.28–33.39)	102.45 (83.57–254.64)	6.29 (4)
			48		1.9 ± 0.29	3.43 (1.09–8.77)	19.83 (9.62–107.14)	4.09 (3)
	PD	2002	24	228	2.0 ± 0.32	19.76 (11.02–33.72)	44.21 (26.02–67.82)	3.75 (4)
			48		1.9 ± 0.39	2.48 (0.929–7.74)	21.92 (12.36–70.26)	7.79 (3)
	LD	2001	24	212	1.9 ± 0.23	6.21 (2.48–9.62)	21.74 (11.36–72.34)	4.98 (4)
			48		2.2 ± 0.28	1.51 (0.856–4.91)	5.21 (1.27–9.75)	2.29 (3)
	LD	2002	24	230	1.9 ± 0.12	9.15 (5.47–23.59)	31.21 (16.13–53.22)	5.88 (4)
			48		1.8 ± 0.09	6.15 (3.58–9.86)	20.78 (14.03–56.69)	2.79 (3)
Riverside	PD	2001	24	225	2.1 ± 0.58	8.11 (5.64–16.76)	62.47 (31.61–116.68)	6.46 (4)
			48		1.9 ± 0.47	2.61 (1.05–6.38)	21.18 (15.47–33.43)	1.83 (2)
	PD	2002	24	231	1.9 ± 0.25	8.51 (3.22–14.64)	56.48 (25.39–104.72)	4.12 (4)
			48		1.8 ± 0.12	1.37 (0.744–4.63)	9.28 (4.57–22.86)	2.53 (3)
	LD	2001	24	215	1.8 ± 0.24	11.34 (6.18–29.76)	99.45 (41.53–215.66)	5.11 (4)
			48		2.1 ± 0.19	5.83 (1.36–10.46)	37.87 (17.23–108.54)	5.52 (3)
	LD	2002	24	230	1.9 ± 0.21	11.37 (6.26–25.49)	120.47 (59.45–262.82)	6.38 (4)
			48		2.0 ± 0.33	3.56 (1.65–5.86)	19.34 (11.28–54.55)	3.05 (3)
Kern	PD	2001	24	226	1.9 ± 0.21	43.78 (31.24–73.34)	197.45 (103.63–282.56)	4.63 (4)
			48		1.8 ± 0.54	20.33 (13.53–32.46)	106.27 ((60.51–239.22)	3.68 (3)
	PD	2002	24	215	1.9 ± 0.11	24.45 (16.34–35.57)	109.63 (65.49–231.58)	4.89 (4)
			48		2.0 ± 0.34	10.58 (6.45–21.91)	48.40 (30.63–111.83)	4.68 (3)
	LD	2001	24	205	2.0 ± 0.29	46.37 (28.81–81.10)	197.24 (112.45–343.63)	4.87 (4)
			48		1.9 ± 0.17	9.11 (5.37–31.45)	26.90 (9.52–59.82)	1.87 (2)
	LD	2002	24	230	2.1 ± 0.43	31.36 (23.58–44.29)	118.33 (73.56–171.09)	5.36 (4)
			48		2.0 ± 0.14	7.27 (4.46–15.83)	18.91 (6.27–22.93)	3.65 (3)

ever, a five-fold increase in toxicity was observed in Kern county group of adults after 48-h exposure, but the responses were not significant. Although a lower LC_{50} value of 31.36 ng/ml was observed for Kern county insects in 2002, no significant differences in responses were observed for any of the populations of *H. coagulata* from the previous year, suggesting that no shifts in baseline data occurred. These results are similar to the baseline data obtained with the petri dish method for dimethoate.

Slope values for dimethoate treatments were low ranging from 1.8 to 2.1 for both techniques in 2001 and 2002, suggesting that the *H. coagulata* populations were heterogenous in their response to the compound.

Cyfluthrin. Comparison of mortality responses of *H. coagulata* populations to cyfluthrin from the three California locations for 2 yr using both techniques is shown in Table 4. In 2001, San Bernardino populations were the most susceptible (LC_{50} = 2.43 ng/ml) to cyfluthrin residues in the petri dish, whereas Riverside county adults were the least sensitive (LC_{50} = 41.17 ng/ml), a 17-fold difference between the two populations. Kern county populations were three-fold less sensitive (LC_{50} = 8.26 ng/ml) in their response to cyfluthrin. Although toxicity increased after 48 h to

Table 4. Comparison of toxicity of cyfluthrin to *H. coagulata* adults by petri dish and leaf-dip bioassays

Location (county)	Test method	Yr	Time	*n*	Slope ± SE	LC_{50} ng/ml (95% FL)	LC_{90} ng/ml (95% FL)	χ^2 (df)
San Bernardino	PD	2001	24	335	1.2 ± 0.21	2.43 (0.413–5.82)	34.82 (10.38–129.10)	4.47 (4)
			48		1.4 ± 0.07	0.404 (0.118–2.29)	10.04 (2.33–57.51)	3.89 (3)
	PD	2002	24	308	1.3 ± 0.61	1.49 (0.716–6.62)	112.24 (22.62–232.82)	5.63 (4)
			48		1.5 ± 0.32	0.091 (0.067–0.452)	1.61 (0.726–5.63)	2.29 (3)
	LD	2001	24	320	1.1 ± 0.10	20.82 (4.29–106.46)	126.85 (55.05–223.11)	8.14 (4)
			48		1.2 ± 0.21	0.354 (0.065–1.38)	12.43 (9.81–32.51)	5.45 (3)
	LD	2002	24	315	1.3 ± 0.33	19.03 (6.26–63.71)	231.27 (70.36–470.23)	7.13 (4)
			48		2.3 ± 0.35	9.17 (2.38–22.65)	28.49 (10.82–52.28)	3.68 (3)
Riverside	PD	2001	24	302	2.5 ± 0.40	41.17 (12.87–73.45)	188.76 (89.63–217.10)	4.22 (4)
			48		2.6 ± 0.34	8.25 (5.18–20.82)	102.37 (51.43–208.62)	3.96 (3)
	PD	2002	24	327	1.2 ± 0.46	19.11 (15.06–131.37)	513.20 (124.19–740.28)	6.36 (4)
			48		1.2 ± 0.09	1.35 (0.301–6.48)	102.32 (12.92–226.05)	3.34 (3)
	LD	2001	24	330	2.0 ± 0.52	88.21 (56.03–145.73)	114.92 (36.23–150.97)	2.96 (4)
			48		1.8 ± 0.30	18.45 (5.39–60.51)	124.18 (92.73–161.22)	3.17 (3)
	LD	2002	24	330	1.4 ± 0.18	28.17 (6.38–98.02	223.41 (165.29–356.02)	5.72 (4)
			48		1.6 ± 0.25	5.26 (2.30–9.72)	31.82 (14.24–105.38)	3.54 (3)
Kern	PD	2001	24	310	1.0 ± 0.34	8.26 (2.10–28.39)	94.21 (56.36–281.32)	5.32 (4)
			48		1.3 ± 0.03	1.61 (0.220–5.62)	25.82 (5.25–102.03)	4.46 (3)
	PD	2002	24	325	1.2 ± 0.16	2.16 (0.623–6.21)	9.28 (2.53–37.58)	3.48 (4)
			48		1.5 ± 0.27	0.154 (0.075–0.584)	6.29 (1.28–50.22)	4.69 (3)
	LD	2001	24	295	1.5 ± 0.11	41.22 (13.28–61.23)	95.61 (28.74–183.02)	5.39 (4)
			48		1.6 ± 0.29	26.28 (16.11–70.20)	67.47 (37.81–105.07)	4.82 (3)
	LD	2002	24	310	1.4 ± 0.20	81.37 (20.53–152.02)	169.22 (104.26–394.10)	6.54 (4)
			48		1.2 ± 0.33	7.15 (1.06–14.25)	53.04 (11.72–102.67)	3.97 (3)

Table 5. Comparison of toxicity of acetamiprid to *H. coagulata* adults by petri dish and leaf-dip bioassays

Location (county)	Test method	Yr	Time	*n*	Slope ± SE	LC_{50} ng/ml (95% FL)	LC_{90} ng/ml (95% FL)	χ^2 (df)
San Bernardino	PD	2001	12	286	2.3 ± 0.27	64.48 (22.56–95.09)	370.11 (264.56–515.29)	4.81 (4)
			24		2.4 ± 0.23	16.55 (3.62–61.34)	144.39 (71.53–294.68)	3.86 (3)
	PD	2002	12	352	1.5 ± 0.34	6.12 (0.963–12.54)	55.83 (11.29–109.47)	5.12 (4)
			24		2.5 ± 0.46	5.51 (2.16–11.30)	44.34 (10.74–91.28)	3.64 (3)
	LD	2001	24	265	1.8 ± 0.24	64.42 (21.81–92.56)	146.90 (78.42–205.60)	3.54 (4)
			48		3.1 ± 0.61	12.55 (8.63–20.39)	21.10 (12.23–48.07)	3.26 (3)
	LD	2002	24	255	1.8 ± 0.24	2.73 (1.12–6.90)	16.55 (8.19–56.26)	3.20 (4)
			48		1.9 ± 0.42	0.652 (0.302–2.05)	7.36 (2.48–15.83)	2.88 (4)
Riverside	PD	2001	24	290	1.7 ± 0.35	0.823 (0.451–2.85)	9.45 (3.72–19.55)	3.53 (4)
			48		2.2 ± 0.41	0.037 (0.024–0.088)	0.462 (0.163–6.15)	3.38 (3)
	PD	2002	24	310	2.0 ± 0.19	0.637 (0.157–0.938)	2.25 (0.519–22.49)	3.86 (4)
			48		2.4 ± 0.28	0.134 (0.860–0.559)	0.912 (0.275–5.53)	3.24 (3)
	LD	2001	24	274	2.6 ± 0.37	8.62 (4.33–15.14)	41.05 (19.48–62.42)	3.85 (4)
			48		2.4 ± 0.46	3.63 (1.22–8.46)	24.27 (9.55–40.92)	4.01 (3)
	LD	2002	24	325	1.6 ± 0.52	5.54 (3.27–9.62)	49.38 (6.48–98.31)	3.65 (4)
			48		1.8 ± 0.29	0.642 (0.157–2.59)	3.71 (1.53–13.65)	3.26 (3)
Kern	PD	2001	24	315	2.8 ± 0.22	166.25 (151.28–231.05)	575.40 (325.62–682.12)	4.21 (4)
			48		2.3 ± 0.34	55.43 (16.56–93.71)	140.26 (127.37–213.64)	3.36 (3)
	PD	2002	24	325	3.9 ± 0.30	338.56 (147.22–374.82)	549.30 (471.23–632.37)	3.77 (4)
			48		1.9 ± 0.28	137.08 (90.15–198.64)	208.44 (145.24–354.11)	3.42 (3)
	LD	2001	24	315	2.0 ± 0.14	436.10 (183.09–561.13)	1,170 (932.21–2,865)	4.85 (4)
			48		1.8 ± 0.28	364.56 (121.52–510.30)	641 (493.22–928.24)	4.11 (3)
	LD	2002	24	320	1.4 ± 0.11	83.62 (17.65–140.47)	306.45 (133.82–523.43)	3.87 (3)
			48		1.6 ± 0.28	6.64 (3.78–21.38)	45.36 (22.45–124.21)	2.66 (2)

cyfluthrin in all three populations, the increase was not significant.

Populations of *H. coagulata* from the three regions did not show significant shifts in response to cyfluthrin with the petri dish technique in 2002 compared with the previous year as indicated by small differences in LC_{50} values (Table 4). Riverside adults remained the least susceptible as in the previous year. In contrast to nonsignificant increases in susceptibility to cyfluthrin after 48-h exposure in 2001, significant increases in toxicity were observed after 48-h exposure in all populations in 2002. Insects from San Bernardino showed a 16-fold increase in toxicity (LC_{50} = 0.091 ng/ml), whereas adults from both Riverside (LC_{50} = 1.35 ng/ml) and Kern (LC_{50} = 0.154 ng/ml) counties were equally more susceptible by 14-fold. These results indicate that *H. coagulata* populations from all three locations were more susceptible to cyfluthrin in 2002.

Adult *H. coagulata* from all three locations were less sensitive to cyfluthrin using the leaf dip technique compared with the petri dish method in 2001 (Table 4). Although San Bernardino adults remained the most susceptible to cyfluthrin as with the petri dish technique, mortality was reduced by nine-fold (LC_{50} = 20.82 ng/ml) after 24-h exposure to cyfluthrin-treated leaves of citrus in the greenhouse. Similar results were obtained by the leaf dip technique for Riverside populations confirming them to be the least susceptible (LC_{50} = 88.21 ng/ml) to cyfluthrin as was demonstrated by the petri dish technique (LC_{50} = 41.17 ng/ml). Kern county populations were again intermediate in their response to cyfluthrin but exhibited a higher LC_{50} (41.22 ng/ml) compared with the toxic value obtained with the petri dish method (LC_{50} = 8.26 ng/ml). Mortality increased significantly by 59-fold (LC_{50} = 0.354 ng/ml) after 48 h exposure by this method in San Bernardino adults compared with only six-fold more sensitivity (LC_{50} = 0.404 ng/ml) with the petri dish technique in the same population. Additionally, in contrast to the sensitive responses of *H. coagulata* from San Bernardino when exposed for a longer period to the compound, no significant decreases in LC_{50} values were observed after 48 h by the leaf dip method in Riverside (five-fold, LC_{50} = 18.45 ng/ml) or Kern county (two-fold, LC_{50} = 26.28 ng/ml) adults.

No dramatic shifts in susceptibility from year to year in subsequent bioassays were recorded with the leaf dip technique in 2002, except in Kern county insects, which were two-fold less susceptible (LC_{50} = 81.37 ng/ml) to cyfluthrin than in the previous year. Toxicity increased with longer exposure in Kern county insects in 2002; unlike the previous year's observations, a significant increase of 11-fold after 48-h exposure (LC_{50} = 7.15 ng/ml). A nonsignificant increase in toxicity occurred after 48-h exposure in insects from both San Bernardino (LC_{50} = 9.17 ng/ml) and Riverside adults (LC_{50} = 5.26 ng/ml).

The slope values determined for the three populations from the probit regression data ranged widely from 1.2 to 3.1 with the petri dish technique. Riverside populations exhibited the highest slope values (2.5–3.1) compared with San Bernardino or Kern county populations. A wide variation also was observed in slope values by the leaf dip technique for all three populations. Riverside populations again showed higher slope values ranging from 1.8 to 3.2 using the leaf dip technique compared with 1.1–2.3 for San Bernardino or Kern county insects, suggesting that the Riverside adults were less heterogeneous in their responses to cyfluthrin. Kern county insects had the lowest slope values indicating more heterogeneity within them.

Table 6. Toxicity of chlorpyrifos against *H. coagulata* adults

Location (county)	Yr	Time	*n*	Slope ± SE	LC_{50} ng/ml (95% FL)	LC_{90} ng/ml (95% FL)	χ^2 (df)
San Bernardino	2001	24	240	2.2 ± 0.23	9.01 (7.21–14.12)	35.43 (24.65–57.64)	3.05 (4)
		48		2.5 ± 0.46	4.26 (2.05–7.17)	17.15 (11.23–27.58)	3.39 (3)
	2002	24	225	1.8 ± 0.16	4.36 (2.62–7.45)	21.08 (15.47–36.33)	3.77 (4)
		48		2.7 ± 0.38	0.835 (0.602–1.87)	4.28 (2.11–7.55)	2.93 (3)
Riverside	2001	24	228	1.7 ± 0.40	3.42 (1.36–5.68)	36.22 (15.46–109.16)	5.30 (4)
		48		1.5 ± 0.22	1.47 (0.746–3.65)	9.51 (2.75–19.39)	1.52 (3)
	2002	24	330	2.3 ± 0.35	1.12 (0.825–3.42)	14.45 (9.08–30.53)	5.12 (4)
		48		2.5 ± 0.16	0.625 (0.406–2.51)	7.63 (3.24–14.48)	2.47 (3)
Kern	2001	24	227	2.3 ± 0.15	1.53 (0.972–3.36)	17.83 (8.43–39.74)	5.24 (4)
		48		2.9 ± 0.22	0.216 (0.152–0.569)	0.928 (0.547–2.38)	2.28 (3)
	2002	24	255	2.2 ± 0.18	1.38 (0.745–5.26)	30.57 (7.36–51.88)	4.36 (4)
		48		2.7 ± 0.46	0.746 (0.415–3.39)	6.58 (4.65–10.18)	2.64 (3)

Acetamiprid. Exposure of *H. coagulata* adults from Riverside to acetamiprid in the petri dish was found to be very lethal as indicated by extremely low LC_{50} values of 0.823 ng/ml at 24 h and a further decrease of 22-fold to 0.037 ng/ml at 48 h posttreatment (Table 5). The concentrations of acetamiprid effective against Riverside insects were well below the recommended field rates. Acetamiprid was 20-fold less toxic to San Bernardino populations (LC_{50} = 16.55 ng/ml at 24 h) compared with Riverside populations. However, adults from Kern County were the least susceptible among the three populations with a high LC_{50} of 166.25 ng/ml (24 h). The difference in toxicity was significantly higher by 202-fold compared with Riverside adults but only by 10-fold to San Bernardino *H. coagulata.* This trend continued through to the following year, which showed that *H. coagulata* from Kern county were again the least susceptible with a two-fold higher LC_{50} of 338.56 ng/ml at 24 h compared with the value observed in the previous year. This resulted in a striking difference of 531-fold decrease in susceptibility compared with Riverside insects, which were the most susceptible (LC_{50} = 0.637 ng/ml at 24 h) in 2002 also. The LC_{50} values in 2002 for the San Bernardino populations (5.51 ng/ml) also decreased, resulting in a greater difference in toxicity (61-fold) compared with Kern county insects.

Clear differences in responses of *H. coagulata* from the three regions of California to acetamiprid were apparent when the exposure was made to the insects by the leaf-dip method under greenhouse conditions. But the same trend in susceptibility responses obtained by the petri dish technique was apparent with the leaf dip technique (Table 5). For example, Riverside populations were the most susceptible to acetamiprid, with the lowest LC_{50} and LC_{90} values (8.62 and 41.05 ng/ml, respectively) recorded using the leaf dip technique. Compared with Riverside adults, moderately higher toxicity of seven-fold (LC_{50} = 64.42 ng/ml) to acetamiprid was observed at 24 h posttreatment in San Bernardino populations. The highest difference in toxicity was again observed between Kern and Riverside county *H. coagulate* of 51-fold as indicated by high LC_{50} values (LC_{50} = 436.10 and 364.56 ng/ml at 24 and 48 h posttreatment) in Kern county insects. Although the difference in toxicity between the latter two populations was not as high as was shown with the petri dish technique (202-fold), these results confirmed that Kern *H. coagulata* were the least sensitive to acetamiprid among the three populations regardless of the technique.

Leaf dip technique tests in 2002 revealed that all three regional populations were more susceptible to acetamiprid than they were in the previous year. Insects from San Bernardino and Kern counties were significantly more susceptible in 2002 compared with the baseline data observed in 2001. Riverside insects remained the most susceptible as in the previous year with the lowest LC_{50} of 5.54 ng/ml. After 48-h exposure, toxicity increased significantly by four-fold in San Bernardino adults and by nine-fold in Riverside insects. A 13-fold increase in toxicity was observed in Kern county adults, but the increase was not significant based on overlapping fiducial limits.

Slopes obtained from the dose–response regression data for acetamiprid using the petri dish technique were fairly high ranging from 1.9 to 3.9. Slopes obtained with the leaf dip bioassay ranged from 1.4 to 3.1 (Table 5).

Baseline Susceptibility to Organophosphates. The baseline data for two organophosphates, chlorpyrifos and dimethoate, were estimated in this study. Results for dimethoate were shown in the section above as determined with two techniques. Baseline data for chlorpyrifos using the petri dish technique are presented below.

Chlorpyrifos. Based on the low LC_{50} and LC_{90} values presented in Table 6, chlorpyrifos was highly toxic to *H. coagulata.* Among the three populations, Kern county insects were significantly more susceptible (LC_{50} = 1.53 ng/ml) to chlorpyrifos in 2001 than the populations from San Bernardino county (LC_{50} = 9.01 ng/ml) (Table 6). Riverside County *H. coagulata* adults also were very sensitive to chlorpyrifos (LC_{50} = 3.42 ng/ml) compared with adults from San Bernardino county. In general, toxicity increased after 48-h exposure in the three populations but only moderately. The LC_{50} decreased by two-fold (LC_{50} = 4.26 ng/ml) in San Bernardino county insects, and only a minimal increase in toxicity was observed for the Riverside county populations. However, toxicity increased significantly by seven-fold (LC_{50} = 0.216 ng/ml) in insects from Kern County. Sensitivity to chlorpyrifos (24-h exposure) did not shift significantly

Table 7. Toxicity of Pyrethroids to *H. coagulata* adults

Compound	Location (county)	Yr	Test time	*n*	Slope ± SE	LC_{50} ng/ml (95% FL)	LC_{90} ng/ml (95% FL)	χ^2 (df)
Bifenthrin	San Bernardino	2001	24	315	1.3 ± 0.18	4.42 (2.19–8.63)	59.18 (26.75–121.08)	6.47 (4)
			48		1.4 ± 0.30	3.08 (1.41–6.68)	34.37 (16.12–89.69)	3.72 (3)
		2002	24	308	1.9 ± 0.36	60.53 (12.28–93.94)	105.47 (72.31–248.82)	4.63 (4)
			48		2.0 ± 0.51	5.78 (0.683–10.49)	24.61 (8.48–56.69)	3.92 (3)
		2003	24	224	2.6 ± 0.28	0.237 (0.094–0.549)	0.929 (0.565–2.86)	2.46 (4)
	Riverside	2001	24	310	1.2 ± 0.23	0.149 (0.045–0.327)	1.54 (0.638–23.46)	3.36 (4)
			48		3.3 ± 0.38	0.046 (0.028–0.069)	0.212 (0.095–0.464)	3.06 (3)
		2002	24	307	1.4 ± 0.19	0.355 (0.093–0.647)	2.72 (0.918–17.56)	3.49 (3)
			48		2.2 ± 0.24	0.073 (0.035–0.266)	0.648 (0.247–2.73)	2.82 (3)
		2003	24	295	3.4 ± 0.66	0.347 (0.156–0.592)	0.612 (0.458–1.57)	3.10 (4)
	Kern	2001	24	312	1.4 ± 0.24	0.548 (0.163–3.82)	6.32 (2.69–40.38)	3.76 (4)
			48		1.8 ± 0.20	0.062 (0.023–0.152)	0.887 (0.318–4.72)	3.49 (3)
		2002	24	320	1.7 ± 0.32	12.56 (8.47–34.65)	53.87 (11.28–84.91)	2.88 (4)
			48		1.9 ± 0.17	5.63 (1.39–10.16)	35.15 (22.81–47.67)	3.21 (3)
		2003	24	285	2.9 ± 0.27	0.126 (0.092–0.362)	0.728 (0.321–1.38)	2.64 (4)
Esfenvalerate	San Bernardino	2001	24	235	1.1 ± 0.12	0.318 (0.092–12.62)	2.54 (0.446–20.38)	3.19 (4)
			48		1.0 ± 0.14	0.092 (0.076–0.871)	0.753 (0.372–1.89)	4.28 (3)
		2002	24	220	1.0 ± 0.11	0.052 (0.016–0.328)	0.219 (0.068–5.62)	3.47 (4)
	Riverside	2001	24	220	1.0 ± 0.13	1.46 (0.462–8.58)	31.02 (8.57–91.29)	4.48 (4)
			48		1.1 ± 0.19	0.348 (0.104–11.85)	6.92 (0.782–24.26)	3.46 (3)
		2002	24	234	1.1 ± 0.10	2.23 (0.902–11.36)	22.51 (9.23–50.56)	3.98 (4)
			48		1.4 ± 0.24	0.812 (0.254–3.28)	3.60 (1.44–11.13)	3.37 (3)
	Kern	2001	24	302	1.2 ± 0.32	17.45 (7.51–44.26)	85.12 (20.16–162.29)	3.81 (4)
			48		1.6 ± 0.19	10.36 (7.02–27.92)	89.20 (19.91–129.54)	3.72 (3)
		2002	24	235	1.1 ± 0.14	92.21 (10.58–181.26)	289.25 (154.52–420.17)	3.43 (4)
			48		1.3 ± 0.31	13.45 (5.64–47.22)	166.46 (69.86–250.03)	3.32 (3)
Fenpropathrin	San Bernardino	2001	24	312	1.0 ± 0.13	72.56 (34.23–149.22)	353.27 (126.34–474.52)	6.25 (4)
			48		1.2 ± 0.22	61.54 (10.32–118.91)	138.59 (52.84–294.34)	5.32 (3)
		2002	24	320	1.0 ± 0.12	26.53 (7.51–170.02)	134.94 (85.73–314.30)	4.87 (4)
			48		1.9 ± 0.26	17.48 (10.39–46.73)	138.44 (76.26–249.38)	4.12 (3)
	Riverside	2001	24	200	1.7 ± 0.11	49.82 (20.39–80.11)	127.03 (48.46–172.64)	3.35 (4)
			48		2.6 ± 0.25	7.53 (4.10–19.26)	26.81 (9.47–42.39)	2.93 (3)
		2002	24	214	1.0 ± 0.11	44.27 (12.28–116.62)	227.63 (93.20–360.72)	3.84 (4)
			48		1.2 ± 0.22	1.24 (0.743–12.56)	36.72 (14.37–101.03)	4.01 (3)
	Kern	2001	24	306	1.2 ± 0.21	63.62 (44.71–204.56)	226.10 (128.82–486.15)	5.82 (4)
			48		1.2 ± 0.32	16.48 (1.83–84.43)	211.07 (48.65–476.22)	4.18 (3)
		2002	24	215	1.1 ± 0.25	20.37 (6.50–59.46)	126.25 (21.59–313.91)	4.76 (4)
			48		1.3 ± 0.18	0.705 (0.428–4.26)	22.19 (7.57–49.12)	3.65 (3)

in the three *H. coagulata* populations when follow-up tests were conducted in 2002 as indicated by the range of LC_{50} values (1.12–4.36 ng/ml) (Table 6). Toxicity increased five-fold in *H. coagulata* from San Bernardino county following a 48-h exposure (LC_{50} = 0.835 ng/ml) but by less than two-fold in both Riverside (LC_{50} = 0.625 ng/ml) and Kern county adults (LC_{50} = 0.746 ng/ml).

The slope values generated from dose–mortality responses to chlorpyrifos varied, ranging from 1.5 to 2.9 in 2001 for the three *H. coagulata* populations (Table 6). Lower slope values were observed in tests against the Riverside population during first-year tests, indicating some heterogeneity among the population compared with more homogeneity in Kern county population based on the higher slope values (2.3–3.2). Insects from Riverside county showed increased slope values (2.3–2.5) the following year.

Baseline Susceptibility to Pyrethroids. Baseline susceptibility data to four pyrethroids against *H. coagulata* were determined in this study. Results for cyfluthrin were presented in Table 4, whereas results for bifenthrin, esfenvalerate, and fenpropathrin are presented below.

Bifenthrin. Table 7 shows comparisons of LC_{50}, LC_{90}, fiducial limits, and slopes of dose–mortality responses of *H. coagulata* from three locations of California to three pyrethroids, bifenthrin, esfenvalerate, and fenpropathrin. The potency of bifenthrin against *H. coagulata* adults was very high as indicated by low LC_{50} values. There were clear differences in the susceptibility responses of the three groups of *H. coagulata* to bifenthrin in 2001. Populations from San Bernardino were the least susceptible (LC_{50} = 4.42 ng/ml) at 24 h posttreatment, and Riverside insects were the most susceptible (LC_{50} = 0.149 ng/ml) with a 30-fold difference in toxicity between the two populations. Kern county adults were in mid-range of susceptibility (LC_{50} = 0.548 ng/ml), eight-fold less than San Bernardino insects and four-fold more than Riverside populations. Toxicity to bifenthrin did not increase significantly in either San Bernardino (LC_{50} = 3.08 ng/ml) or Riverside (LC_{50} = 0.046 ng/ml) county populations with longer exposure, but toxicity in Kern county adults (LC_{50} = 0.062 ng/ml) increased by nine-fold after 48 h of exposure.

The LC_{50} values for bifenthrin were significantly higher in 2002 for all populations. Although the rela-

tive susceptibility to bifenthrin decreased significantly in all populations, results revealed the same trend as in the previous year. Adults from San Bernardino County were the least sensitive to bifenthrin and insects from Riverside were the most sensitive. Susceptibility decreased by 14-fold (LC_{50} = 60.53 ng/ml) in adults from San Bernardino in 2002. Adults of Kern county were also significantly (23-fold) less sensitive in 2002. Riverside populations showed a small nonsignificant decrease in susceptibility (LC_{50} = 0.355 ng/ml) to bifenthrin. Toxicity increased significantly in adults from San Bernardino county by 10-fold after 48-h exposure. Toxicity to bifenthrin against the three populations also was examined in 2003. Surprisingly, unlike the two previous years, no marked differences were observed between the three populations at 24 h exposure as indicated by the range of LC_{50} values of 0.126–0.359 ng/ml.

Slopes of concentration–response regression varied widely in the three populations with the highest slopes exhibited in data from 2003. The slope values ranged from 1.1 to 3.4. The lower slope values recorded in previous years may indicate a higher degree of heterogeneity among the three populations that seems to have decreased by 2003.

Esfenvalerate. The LC_{50} values among the different populations to esfenvalerate were determined to be significantly variable by the petri dish technique (Table 7). In contrast to the baseline data for bifenthrin, populations from San Bernardino county were the most susceptible (LC_{50} = 0.318 ng/ml) to esfenvalerate, whereas insects from Kern county were the least susceptible (LC_{50} = 17.45 ng/ml). A significant sensitivity difference of 55-fold was determined between the two populations. Adults from Riverside were intermediate in their response to esfenvalerate (LC_{50} = 1.46 ng/ml). A small increase of two- to four-fold in toxicity to esfenvalerate was recorded in all populations after 48-h exposure.

In 2002, *H. coagulata* populations from the three locations showed a similar trend in responses to esfenvalerate. As in the previous year, San Bernardino populations were the most susceptible at an LC_{50} of 0.052 ng/ml (six-fold increase in susceptibility), whereas Kern county insects were the least susceptible at an LC_{50} of 92.21 ng/ml (seven-fold decrease in susceptibility). Most of San Bernardino adults did not survive after 24 h exposure in the petri dish and hence no LC_{50} was determined for 48-h exposure. Insects from Riverside also were very sensitive to esfenvalerate (LC_{50} = 2.23 ng/ml) (Table 7). Toxicity increased significantly in insects from Kern County to esfenvalerate by seven-fold after 48 h posttreatment.

The slope values generated from dose-mortality response to esfenvalerate did not vary widely among the three populations of *H. coagulata.* The slopes were fairly flat in general, ranging from 1.0 to 1.3, indicating greater heterogeneity among these populations in their responses to esfenvalerate compared with bifenthrin.

Fenpropathrin. Dose–response mortality data to fenpropathrin determined with the petri dish are shown in Table 7. No marked differences in responses of *H. coagulata* from the three locations were observed, as indicated by LC_{50} values in 2001. Compared with the potency of bifenthrin and esfenvalerate against *H. coagulata* from the three locations, sensitivity to fenpropathrin was less, as indicated by higher LC_{50}'s (ranging from 49.82 to 72.56 ng/ml). Additionally, toxicity to fenpropathrin did not increase significantly over time against adults from San Bernardino or Kern counties (48 h), but it did increase to Riverside populations significantly by seven times (LC_{50} = 7.53 ng/ml).

Bioassay results in 2002 did not show any significant shifts toward increases in LC_{50} values at 24 h posttreatment in any of the three populations (Table 7). However, toxicity did increase significantly over time in both Riverside (36-fold) and Kern county (29-fold) adults but not in San Bernardino county insects.

The slope values based on dose–mortality responses of *H. coagulata* populations to fenpropathrin were similar to values generated for esfenvalerate. Slopes were low from 1.0 to 2.0 with the exception of 2.6 for Riverside, suggesting less heterogeneity than in San Bernardino and Kern county populations.

Baseline Susceptibility of Neonicotinoids. Baseline data of *H. coagulata* to three neonicotinoids—acetamiprid, imidacloprid, and thiamethoxam—was established in this study. Toxicity data to acetamiprid was determined by two techniques and is presented in Table 5. Susceptibility of *H. coagulata* adults to imidacloprid and thiamethoxam expressed as LC_{50} and LC_{90} values are shown in Table 8.

Imidacloprid. Responses of *H. coagulata* populations from the three California regions were variable to imidacloprid when exposed systemically (Table 8). Bioassay results in 2001 showed imidacloprid to be highly toxic to adults from San Bernardino county, as shown by a low LC_{50} (5.06 ng/ml in 24 h). Although, insects from both Riverside and Kern counties exhibited higher LC_{50} values of 1,040 and 1,272 ng/ml compared with San Bernardino insects, which translates into toxicity that was greater by 205- and 251-fold, respectively, both populations also were highly susceptible to imidacloprid after longer exposure. Toxicity increased variably at 48 h posttreatment in *H. coagulata* adults from Kern and Riverside county, by 24 times in Kern county adults, whereas a significantly higher increase of 60-fold was exhibited in Riverside populations. These results show significant geographic variation in responses to imidacloprid in the establishment of initial baseline data.

Systemic bioassays conducted during the following year (2002) showed that all populations of *H. coagulata* were more susceptible to imidacloprid, similar to *H. coagulata* responses to acetamiprid. San Bernardino county adults were the most susceptible as in the previous year with a moderate increase in susceptibility (four-fold), whereas insects from Kern county remained the least sensitive to imidacloprid (LC_{50} = 361.34 ng/ml), although an increase in toxicity was evident compared with 2001. A significant difference of 289-fold in sensitivity was observed between San

Table 8. Toxicity of two neonicotinoids to *H. coagulata* adults

Compound	Location (county)	Test method	Yr	Test time	*n*	Slope ± SE	LC_{50} ng/ml (95% FL)	LC_{90} ng/ml (95% FL)	χ^2 (df)
Imidacloprid	San Bernardino	Uptake	2001	24	255	1.1 ± 0.19	5.06 (0.627–31.73)	56.29 (10.58–141.70)	3.94 (4)
				48		1.0 ± 0.20	3.52 (0.772–31.40)	39.08 (6.12–108.19)	4.24 (3)
			2002	24	285	1.2 ± 0.21	1.25 (0.750–8.25)	9.53 (1.47–32.82)	3.59 (3)
				48		1.1 ± 0.34	0.087 (0.014–0.858)	2.63 (0.910–19.39)	3.10 (3)
	Riverside	Uptake	2001	24	255	1.1 ± 0.48	1,040 (752.10–1,562)	4,124 (2,861–6,917)	5.25 (4)
				48		1.1 ± 0.18	17.34 (11.57–38.11)	89.06 (23.48–231.04)	4.32 (3)
			2002	24	306	1.2 ± 0.21	24.71 (5.90–93.38)	256.06 (41.39–424.51)	3.98 (4)
				48		1.2 ± 0.34	8.34 (0.472–52.24)	88.53 (14.62–177.09)	4.11 (3)
	Kern	Uptake	2001	24	312	1.1 ± 0.30	1,272 (678.02–2,536)	2,156 (685.17–3,598)	6.24 (4)
				48		1.2 ± 0.22	53.09 (11.46–109.21)	135.60 (62.55–265.18)	4.57 (3)
			2002	24	295	1.2 ± 0.35	361.34 (92.86–510.22)	1,320 (785.33–1,724)	4.76 (4)
				48		1.2 ± 0.45	24.36 (8.62–121.23)	156.12 (128.20–311.45)	3.74 (3)
Thiamethoxam	San Bernardino	PD	2001	12	330	1.5 ± 0.39	9,271 (5,934–11,773)	19,412 (14,933–31,391)	9.25 (4)
				24		1.9 ± 0.61	1,012 (522.33–1,890)	4,778 (2,410–6,549)	5.21 (3)
		PD	2002	12	310	1.8 ± 0.22	3,762 (1,995–5,034)	19,782 (10,350–23,183)	6.72 (4)
				24		2.0 ± 0.43	1,114 (544.90–2,240)	4,954 (2,405–10,726)	5.46 (3)
	Riverside	PD	2001	12	326	1.1 ± 0.12	3,083 (1,843–4,966)	38,252 (21,024–49,510)	7.48 (4)
				24		1.6 ± 0.24	239.20 (106.22–462.07)	1,296 (915.40–4,592)	5.25 (3)
		PD	2002	12	330	1.5 ± 0.35	1,834 (1,211–2,805)	7,299 (2,735–9,322)	3.88 (3)
				24		1.7 ± 0.19	330.65 (172.05–517.34)	1,802 (1,163–4,942)	4.63 (3)
	Kern	PD	2001	12	326	2.1 ± 0.36	2,797 (1,564–5,018)	8,603 (5,741–11,506)	3.75 (4)
				24		2.2 ± 0.27	1,866 (1,183–2,059)	6,548 (4,307–8,942)	4.24 (3)
				48		1.7 ± 0.16	644.26 (367.60–911.22)	3,501 (2,874–6,940)	3.98 (3)
		PD	2002	12	320	2.1 ± 0.32	2,963 (2,090–4,231)	6,123 (3,783–8,259)	3.86 (4)
				24		1.7 ± 0.13	2,123 (1,486–3,275)	5,611 (4,717–7,292)	4.12 (3)
				48		2.7 ± 0.13	704.45 (442.80–915.02)	1,206 (913.20–1,684)	3.63 (3)

Bernardino and Kern county adults. Riverside county insects also were more susceptible to imidacloprid by 42-fold in 2002. Toxicity increased in 48 h because of the residual action of imidacloprid in all populations of *H. coagulata.* The slopes of the regression lines were flat in all populations suggesting high genetic heterogeneity in the natural populations.

Thiamethoxam. Based on preliminary results with thiamethoxam, observations of *H. coagulata* mortality were determined to include 12, 24, or 48 h posttreatment (Table 8). For the 12-h interval, values at the median lethal concentration were the highest for all *H. coagulata* populations with the San Bernardino insects showing the highest LC_{50} (9,271 ng/ml). However, toxicity increased over time in all populations. Toxicity increased after 24 h to thiamethoxam and showed differences of four- to eight-fold among the three populations (LC_{50} values ranging from 239.20 to 1,866 ng/ml). The most susceptible group to thiamethoxam was the Riverside population (LC_{50} = 239.20 ng/ml at 24 h). Mortality increased rapidly after 24 h, and hence no data were recorded at 48 h posttreatment for San Bernardino or Riverside *H. coagulata* populations. Insects from Kern County were still alive after 24-h exposure, allowing mortality observations to continue to include 48 h posttreatment responses at which time toxicity increased by three-fold. Bioassays conducted subsequently in 2002 revealed no major shifts in susceptibility from year to year.

The slope values determined for thiamethoxam were fairly low, ranging from 1.1 to 2.1. Lower slope values are not atypical for field populations especially in the case of newer compounds with no previous exposure. The slope values did not change in subsequent tests in 2002.

Discussion

Comparison of Bioassay Techniques to Establish Baseline Toxicity. Bioassay methods should be developed to establish data on the initial sensitivity of a pest to existing compounds before new compounds are brought into use (Brent 1986). Although not all compounds in our study tested against the *H. coagulata* were new, it was important to establish sensitive bioassay methods to assess toxicity of various insecticides representing different chemistries early in the chemical management of this pest. Estimating insect mortality by bioassays with treated leaves and/or plants provides valuable and timely information as opposed to extensive field experiments. Initially, two bioassay methods, the petri dish and leaf dip bioassays, were evaluated to identify the most suitable technique to establish baseline susceptibility data. The four candidate insecticides selected for comparison of toxic values by the two methods represented four major chemistries currently in use against pests on citrus and grapes, including *H. coagulata.* Dose–response relationships were established with both techniques and expressed as LC_{50} and LC_{90} values. Bioassay technique and exposure duration influenced the toxic value of each compound. Results showed similarities and variabilities in toxic values between the two techniques among the three *H. coagulata* populations from different regions. For example, application of endosulfan by both techniques resulted in toxic values that were close with overlap of confidence limits for each *H. coagulata* population during the first year. However, significant differences in susceptibilities to endosulfan were observed the following year in two populations with the petri dish technique but not with

the leaf dip method. This difference may be related to sensitivity of the technique. Different bioassay techniques may show responses of different magnitude (Dennehy et al. 1983, McCaffery et al. 1988, Prabhaker et al. 1996). Insects held in clip cages for the leaf dip technique may have been exposed to lower amounts of the compound because of ventilation, compared with enclosed conditions in the petri dish technique. Insects from the three locations also responded similarly to dimethoate with no significant differences in toxic values by either technique. In contrast, clear toxicity differences between geographically isolated populations of *H. coagulata* to acetamiprid were observed by both techniques, although the LC_{50} values were significantly different from year to year. In other examples, concentrations that affected 50% mortality for one population by one technique differed in percentage of mortality for the same population by the second technique. For example, *H. coagulata* adults from San Bernardino county were extremely susceptible to cyfluthrin, exhibiting low toxic values (LC_{50} = 2.43 ng/ml) as determined by the petri dish technique. Similarly, bioassays using the leaf dip technique showed that the same population was the most susceptible to cyfluthrin but exhibited a higher toxic value (LC_{50} = 20.82 ng/ml). One of the reasons for difference in results between the two techniques may be because of environmental influences. Tests were conducted in a greenhouse for the leaf dip bioassay, whereas petri dish bioassays were done in the laboratory. Conditions in the greenhouse are subject to less constant temperatures and humidity. This in turn could have an influence on the behavior of insects related to feeding or avoidance of the chemical, possibly leading to underestimation of *H. coagulata* responses to certain insecticides. Conducting both tests in an environmental chamber with more accurate and constant control of environmental conditions may help eliminate most of the observed variations between the two techniques.

Results of the study presented above indicate that in spite of the differences between techniques in some cases, tests with both petri dish and leaf dip techniques are useful for testing adult responses to contact insecticides. Either test can provide an earlier diagnosis of resistance in *H. coagulata* adults and nymphs through continuous monitoring for susceptibility shifts. The generally small differences in sensitivity observed in the current study are unlikely to present any significant obstacle to their usefulness in management programs. Both techniques had the benefit of producing susceptibility results within 24 and 48 h and were sufficiently sensitive to detect toxicity at extremely low insecticide amounts. The advantage of using either technique is that both adults and nymphs can be tested for susceptibilities to chemicals (N.P., unpublished data). Susceptibility data of immatures is also necessary to determine shifts in sensitivity responses of this pest toward insecticides, because all stages are affected by pesticide applications.

Use of the leaf dip method, however, presented some disadvantages compared with the petri dish method. Treatment of attached citrus leaves by dipping, and insect handling procedures in the greenhouse were time-consuming. Insects also fed more on the attached treated leaves as indicated by significantly higher amounts of excreted water, unlike the behavior in petri dishes in which the output of excreted water was minimal. The high output of water excreta was a potential disadvantage to complete bioassay tests with the leaf dip technique because *H. coagulata* adults were trapped in the excretions that led to higher mortality unrelated to insecticide treatments. There was also a tendency of ants to enter the clip cages and attack the enclosed *H. coagulata.* As a result of these two problems, bioassays were repeated numerous times to avoid mortality errors. An advantage of using the leaf dip method was the absence of insecticide fumigation effects because of ventilation through the clip cages unlike the petri dish technique.

Based on a comparison of baseline data obtained by both petri dish and leaf dip evaluations of four insecticides, the petri dish bioassay consistently demonstrated the efficacy of contact insecticides against all *H. coagulata.* This technique showed geographical treatment differences, even following a short exposure time of 24 h. The petri dish method also was preferable for testing efficacy against adults because it allowed rapid evaluation of a number of insecticides with simple, basic equipment and standardized handling procedure. Therefore, the petri dish technique was selected for subsequent tests to evaluate toxicity of various contact insecticides to establish baseline susceptibility data. One disadvantage of this technique may be the potential fumigation effect on mortality in the enclosed petri dish. Such an effect was observed using an enclosed vial technique for resistance monitoring of whiteflies that could have resulted in a lower estimate of resistance (Prabhaker et al. 1996). However, in the current study with *H. coagulata,* similar LC_{50} values obtained for certain insecticides using both techniques suggest that higher mortality may not have occurred.

The systemic activity of imidacloprid was measured using a modified bioassay technique originally developed for testing whiteflies (Prabhaker et al. 1997, 2005). Variation observed in responses to imidacloprid in the three *H. coagulata* populations from different regions suggests this technique is a feasible bioassay method for routine monitoring that is sufficiently sensitive to detect differences in susceptibilities of *H. coagulata* populations.

Slope values can be a measure of sensitivity of insects' responses to insecticides. A high slope value of the dose–mortality regression data indicates a high degree of sensitivity and correlation between insecticide concentration and mortality (Georghiou and Metcalf 1961). The slope values with both petri dish and leaf dip methods were high for certain compounds but not for others, suggesting that this parameter is also dependent on the chemical used and not just the technique.

Establishment of Baseline Data to Various Insecticides. Generally, concentration–mortality data need to be determined for establishment of a baseline for commonly used insecticides. Once the basic toxicity data have been determined for various compounds against a pest, detection of proportions of resistant individuals in a population is made possible by use of a discriminating dose (Roush and Miller 1986). Our results presented in this study have determined initial baseline toxicity data against *H. coagulate*, and based on this information, a diagnostic dose can be selected to identify proportions of resistant individuals in future resistance monitoring programs

Comparisons of susceptibilities of *H. coagulata* to various insecticides have shown considerable interregional variation to some insecticides. Year-to-year variation in susceptibility also was evident, but this variation was not biased toward decreased susceptibility. Adult *H. coagulata* did not show much variation in their responses to endosulfan during the first year. The following year, however, two of the three populations (San Bernardino and Kern counties) were observed to be significantly more susceptible, whereas insects from Riverside were four times less sensitive. The apparent increased susceptibility in the two populations the following year may be related to a slow adaptation of one or more defense systems in *H. coagulata* originating from different locations having no exposure to endosulfan. Also the differences in LC_{50} values observed from year to year may be because of natural variation in tolerance, especially at the regional level. The two organophosphates, chlorpyrifos and dimethoate, were highly toxic to *H. coagulata* but did not exhibit the same effect on each population. For example, Kern County populations were the most sensitive to chlorpyrifos in 2001, whereas the same population was the least susceptible to dimethoate. The variations in responses of *H. coagulata* to these various insecticides in some cases were likely because of different histories of exposure in each region. Also the mode of action may not be different for the two organophosphates but the mode of absorption might vary for each compound based on the method of exposure. Perhaps behavior of *H. coagulata* involving feeding and avoidance of insecticides also may be related to toxicity, but at present, relationships of *H. coagulata* behavior to insecticide exposure are poorly understood.

Pyrethroids have been remarkably effective as contact insecticides against agricultural pests as well as household pests (Casida and Quistad 1998). Our results demonstrated the high efficacy of pyrethroids against *H. coagulata* from different regions. Both bifenthrin and esfenvalerate were rapid in their action with the maximum effect seen 4–8 h after treatment. Some of the toxicity symptoms observed with pyrethroid treatments included hyperactivity and body tremors. There was no recovery of the affected insects, suggesting that pyrethroids were extremely effective. Adults from San Bernardino County were the least susceptible to bifenthrin among the three populations in 2001. Cyfluthrin also was very effective against *H. coagulata* but less active compared with bifenthrin and esfenvalerate, although residual activity was significantly higher after 48-h exposure. Toxicity to esfenvalerate was consistent among the three populations. Adults from San Bernardino were determined to be the most susceptible, whereas Kern County insects were the least sensitive. But of more significance were the large differences in responses (from 56- to 1,773-fold) detected between San Bernardino and Kern county *H. coagulata.* Such significant inter-regional variations between *H. coagulata* populations to the pyrethroids may be related to earlier indirect exposure of certain insecticides, especially pyrethroids targeted for other citrus pests.

Insects responded variably to each compound in the neonicotinoid group. The effect of imidacloprid on *H. coagulata* was different compared with the pyrethroids. The insects were immobilized slowly with the maximum effect occurring after 24 h. Geographical variations also were observed among the three populations to this group of insecticides. Adult *H. coagulata* from Riverside were more susceptible to acetamiprid and thiamethoxam, whereas San Bernardino insects were the most susceptible to imidacloprid. In spite of differences in responses to neonicotinoid insecticides among two of the populations, Kern County *H. coagulata* were consistently less sensitive to each neonicotinoid as shown by higher LC_{50} values. Although the differences in sensitivity among populations in a laboratory bioassay to insecticides vary significantly, these differences may not necessarily translate to reduced efficacy in the field (Denholm et al. 1984). The systemic properties of this important new chemistry have been exploited very effectively in the management of *H. coagulata* in both citrus orchards and vineyards (Castle et al. 2005). Results of this study confirm the highly toxic nature of the various compounds within this class at extremely low treatment concentrations against geographically distinct populations of *H. coagulata.* These results are not surprising because despite heavy use of imidacloprid for a decade as soil applications to control whiteflies on multiple crops in Imperial Valley and Yuma, resistance to imidacloprid or related compounds has not been reported as a limiting factor (Prabhaker et al. 2005).

Although considerable variation in susceptibility to insecticides was observed by both techniques, all insecticides tested were shown to have great potential for controlling this pest. These results are encouraging because they indicate the absence of cross-resistance between different chemistries. Differences in responses observed may be caused by natural variation within field populations of *H. coagulata* not related to resistance, because all insecticides evaluated as shown were extremely toxic at low rates. Differences also may be related to insect behavior in terms of avoidance of a chemical or reduced feeding on treated leaves. Additionally, *H. coagulata* populations have little or no history of insecticide exposure before their recent rise to serious pest status in California. Resistance was defined by Sawicki (1987) as a genetic

change in response to selection by toxicants that may impair control in the field. Dramatic elevations in LC_{50} values were not observed in subsequent tests the following year in populations from all three regions. Studies have shown that tolerance or resistance to insecticides increases because of heavy selection pressure, multiple generations, lack of refugia, limited immigration, and other ecological plus operational factors (Georghiou and Taylor 1977a, b). However, for *H. coagulata* with only two or three generations annually, combined with less intensive insecticide use patterns to control this pest, development of resistance to insecticides will likely be slow. But laboratory bioassays of insecticides on field populations are essential for documentation of baseline susceptibility data with continued monitoring of *H. coagulata* populations to show major or minor differences among field populations.

Although increased mortality because of longer exposure (48 h) of certain insecticides in this study may be a basic function of experimental exposure time, it was not detectable for other compounds such as chlorpyrifos and dimethoate, two organophosphates. However, residual activity for certain pyrethroids, including cyfluthrin, esfenvalerate, and fenpropathrin, was detectable at 48 h, thus showing increased mortality over time. A significant increase in mortality of 36-fold was detectable to fenpropathrin in *H. coagulata* from Riverside County. Activity of certain compounds can be slower than others; therefore, mortality checks after 24 h may be necessary to evaluate insecticide effectiveness. In general, the various insecticides produced a similar pattern of effects at different time intervals, although the concentration of insecticide associated with a particular level of efficacy varied clearly between insecticides as expected.

The data reported in this study on geographic variation of baseline susceptibility of *H. coagulata* populations from California to conventional as well as the newer neonicotinoid insecticides can be a useful database for continued monitoring. It is important to know the geographic differences in baseline data to specific classes of insecticides so they can be encouraged or avoided as necessary. The baseline data generated also indicated that of the various chemistries that were evaluated, including older conventional and newer reduced risk compounds, the neonicotinoids show promise as effective tools for control of *H. coagulata* in California. Use of chemicals with different modes of action can help decrease the selection pressure for evolution of resistance. Therefore, the need for greater diversity of chemicals for *H. coagulata* control should be emphasized based on the results of this study.

In summary, results presented in this study have provided a baseline database on susceptibility of *H. coagulata* to a number of insecticides. Information presented here combined with continuous monitoring will enable a better understanding of the dynamics involved in evolution of insecticide resistance in this newer pest. Successful continuous monitoring of shifts in susceptibility after establishment of baseline data for a new pest is integral to any resistance management program that provides practical information from which selection and use of the most appropriate insecticide can be made. The petri dish bioassay technique can be a very feasible method that can be used in future resistance monitoring programs as a standardized technique. It can enable evaluation of suitable resistance management strategies for *H. coagulata* in the event of resistance development.

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